THE ACTIVE PRINCIPLE OF *ERYTHROPHLOEUM LABOUCHERII.*

By James M. Petrie, D.Sc., F.I.C., Linnean Macleay Fellow of the Society in Biochemistry.

(*From the Physiological Laboratory of the University of Sydney.*)

(Plates xxiii.-xxiv., and two Text-figures.)

Erythrophloeum Laboucherii is the Ironwood tree of the Northern Territory and Queensland. It was first described by Baron von Mueller (2), in 1859, under the name of *Laboucheria chlorostachya,* and subsequently as *E. chlorostachys* (F. v. M.) Baillin, and *E. Laboucherii* F. v. M. The latter is the name used in Bentham's Flora Australiensis, in the Index Kewensis and Engler's Botany (1).

This tree is a member of the family Leguminosae and is often referred to as Leichhardt's leguminous ironbark tree, or the ironwood tree. Besides the original latin description by von Mueller, the leaves, flowers and pods are figured in the same author's work on Australian Acacias (3) and reproduced, with further notes, in the Queensland Agricultural Journal by F. M. Bailey (7). It is also described in the Queensland Flora (5) and in Bailey's Weeds and Poisonous Plants of Queensland, with an illustration (6). (*See* Plate xxiii.)

Planchon, in 1907, described with great detail the comparative anatomy of *E. chlorostachya, E. guineense, E. couminga,* and *E. Fordi,* in a French publication, and a full abstract of this (9) may be seen in Just's Jahresbericht for 1908.

At the beginning of an investigation of this kind where much time and labour may be expended on one particular plant, it is of importance to have some general knowledge of the genus to which it belongs. In many instances information of much value is obtained by a consideration of the position of a plant relative to the other species, and by a knowledge of the prominent characteristics, or of any remarkable properties which may have been recorded for other members of the genus. With this object the following data have been collected regarding the world distribution of the genus *Erythrophloeum.*

This genus appears to be confined to the Old World, and 12 species are known. In the great forests of Central Africa grows the dreaded ordeal tree of the pigmies, *Erythrophloeum guineense,* and all parts of this tree have long been known to contain a very poisonous alkaloid, named erythrophleine.

Three other species belonging to tropical Africa are *E. purpurascens* Chev., *E. ivorense* Chev., and *E. pubistanineum* Henn., but the nature of their juices is unknown.

In Madagascar is found *E. couminga* Baill.; then crossing into Asia, we find in Further India and China five different species, *E. Fordi* and four others.

Descending to the Philippine Islands one species is met with, *E. densiflorum* (Elmer) Merrill. This plant has been investigated chemically in 1917 by Brill and Wells (20), who found, however, that it contained no poisonous substance. In this species the alkaloid of *E. guineense* was proved absent.

On continuing southwards we meet with the single Australian species *E. Laboucherii,* in Northern and Tropical Australia.

The powerful and poisonous alkaloid, which was first discovered in *E. guineense* of Central Africa, has since been identified also in *E. couminga* of Madagascar, and *E. Fordi* of China. It was proved absent in the Philippine species, and all the others are quite unknown.

With regard to the Australian species the writer has found no record of any experimental work having been done. The Botanist Baron von Mueller is quoted in "Wittstein" (4) as having said that an alkaloid was present in this plant, but beyond this single statement there is no reference to any evidence for its support, and in all probability the remark is founded on analogy alone.

E. Laboucherii is endemic in Northern Australia. It has been found along the whole vast stretch of coast land beginning about Vansittart Bay, the most northerly part of Western Australia, where it was first collected by Allan Cunningham. It continues through the Northern Territory, and flourishes in abundance up the Victoria River and the Roper and Endeavour Rivers, where it was observed by Banks and Solander. It grows on all the great rivers which flow into the Gulf of Carpentaria, and was collected by Robert Brown on the numerous islands in the Gulf. It grows richly in the York Peninsula and is still very plentiful down the east coast of Queensland to the Tropic of Capricorn. Dr. Shirley states that south of Rockhampton it occurs only very sparsely, and rapidly disappears. It is exceedingly rare to find it in south-east Queensland. On Plate xxiv. are photographs of a tree and a close view showing the characteristic bark.

Many Australian trees have been designated ironwoods, but here the name of ironwood tree is peculiarly appropriate, for the red-coloured wood is exceedingly hard, and is probably the hardest of all Australian timbers.

It was used by the aborigines for making their womerahs and spear-heads.

Though this plant was collected by the great Botanists in the early days of Australian Settlement and described in detail by Mueller in 1859, there appears no account of its poisonous properties until comparatively recent years.

F. M. Bailey has described the tree as one of the worst poisonous plants of Queensland, and how large numbers of stock are yearly lost from eating its leaves. He also described how the tree forms numerous leafy shoots near the ground by sprouting from the roots. These masses of young green foliage, therefore, are very accessible to sheep and cattle, and are the chief cause of mortality.

Ten thousand sheep were lost on Cambridge Downs through eating the leaves of this tree. The Stock Inspector who reported the above tremendous loss stated that two leaves were sufficient to kill a goat (7).

Bennett, in 1904, wrote that this plant had proved most disastrous to the camels imported to carry the copper ore from Mt. Garnet (8).

Mr. Meston, formerly Protector of Aborigines of North Queensland, wrote in 1909 that " the bark, wood, leaves, fruit and flowers of this tree are deadly poisonous. Its peculiar property is that it absolutely destroys the optic nerve and one bean mixed in your food would make you totally blind. A splinter from the tree needs about the same treatment as snakebite" (10). It is employed by the natives for criminal purposes.

Mr. Allen, Director of the Botanic Gardens of Darwin, writes that this tree has been very troublesome of late, many sheep, horses, and cows having died from eating the young leaves.

THE ALKALOID OF THE CENTRAL AFRICAN SPECIES: *Erythrophloeum guineense.*

Erythrophloeum guineense is one of the group of poisonous plants used by the pygmies in Central Africa in the preparation of their arrow poisons. It is well known too from its use for criminal purposes. It is the *Nkasa,* the "doom tree" of the West African natives, who use it in the trial by ordeal to detect persons accused of sorcery and witchcraft or other crimes. The bark alone is used for this purpose. A small piece is removed and pulverised; an infusion of this is made, and the accused persons are forced to drink a certain quantity. The general result of drinking this infusion is a rapid appearance of symptoms of poisoning. The first stage is characterised by violent vomiting, but, if a small dose only has been given, these symptoms may disappear and the person recover, in which case he is declared innocent and set free. If, however, a larger dose has been administered, the second stage is rapidly reached in which the vomiting and purging continue, all power is suddenly lost from the limbs and the person falls to the ground. He is then considered guilty of the crime and is either at once put to death, or quickly dies of heart-paralysis, the effect of this powerful draught.

A vivid description of a trial by ordeal in the Gold Coast is to be found in the Pharmaceutical Journal for 1856, where it may be read with all its gruesome details (11).

The ordeal tree was first examined chemically in 1876 by Gallois and Hardy of Paris (12). Their first experience while working with this material was the violent fits of sneezing produced by the powdered plant, and great care had to be taken to prevent the dust penetrating the respiratory passages. An intensely poisonous alkaloid was isolated which was named "erythrophleine" by the authors. Neither the base nor its salts could be obtained in crystalline form, and the alkaloid was found in the bark, leaves and seeds. It was stated to be a powerful heart-poison.

During the same year, E. Merck, of Darmstadt, prepared a large quantity of erythrophleine, and this was distributed to pharmacologists in various countries. From this material Lauder Brunton (13) made a very extensive examination of the physiological properties of the alkaloid. The chief conclusion arrived at was that erythrophleine possessed the pharmacological properties of both the digitalis and the picrotoxin groups in its action on animals. He emphasised also the effects on the respiratory organs: "All the men employed by us in grinding or pounding the bark suffered severely from the violent and irresistible fits of sneezing which attacked them, and in one instance these were accompanied by great faintness and tendency to syncope."

In 1888 there was a large production of published papers in medical literature on the use of erythrophleine as a therapeutic agent. It was employed in heart diseases as a cardiac tonic. It was said to have no cumulative action and therefore, a valuable substitute for digitalis. It was found to have a considerable anaesthetic action like cocaine. It possessed certain local irritant properties when injected subcutaneously or instilled into the eye.

In 1895 Merck succeeded in preparing a new supply of alkaloid, which he purified with extreme care, and with this, Harnack, in 1895 (16), proved that the picrotoxin action was eliminated, and the pure digitalis action alone was exhibited. Even with this pure substance the alkaloid was amorphous and its salts could not be crystallised.

Harnack determined the approximate composition of the base from the analysis of its amorphous platinum salt, and from these results the formula was provisionally expressed as $C_{28}\ H_{43}\ O_7\ N$.

Power and Salway (19) in 1912, from bark collected in the Belgian Congo, isolated an alkaloid which agreed in its properties and composition with that of Harnaek. These two authors worked also on a commercial specimen obtained from Merck. Merck prepared the hydrochloride salt with dry gas: the salt was obtained in the form of a viscid oil and not as before in the amorphous solid state (18). This hydrochloride gave the formula $C_{28}H_{43}O_7N.HCl$ with 6.9 % Cl (19).

The free alkaloid erythrophleine was obtained by precipitating the aqueous solutions of its salts by alkalies. The first floeculent precipitate soon collected into a resinous mass, and finally changed to a thick yellow syrup. It was very soluble in alcohol, ether, ethyl acetate and dilute acids, but insoluble in petroleum spirit or benzene. The substance was very easily decomposed, evaporation in a solution which was not quite neutral being sufficient to change it partly. When heated with acids or alkalies, the solution became yellow, then a brown resinous substance separated, which cooled to a hard brittle solid. This was erythrophleic acid, $C_{27}H_{40}O_8$. It was nitrogen-free, and nearly insoluble in water and acids. It was soluble in alcohol, ether and alkalies. When erythrophleine was heated in this way for a short time only, the nitrogen-free acid which was obtained reduced Fehling's solution, but when the heating was continued to complete destruction of the alkaloid there was no reduction. The reducing was, therefore, due to some intermediate product, which was gradually destroyed. This decomposition was also accompanied by the evolution of a volatile base, which, in the case of material from certain sources, was a nicotine-like base called maneonine, and with that obtained from other sources was shown to be methylamine.

Experimental.

The problem presented is to determine by practical experiment whether the Australian tree, *Erythrophloeum Laboucherii*, owes its poisonous properties to the presence in it of some definite chemical compound like the alkaloid of *E. guineense*, and whether such an alkaloid, if present, is identical with the erythrophleine isolated from that plant.

The material for this investigation was kindly offered by Mr. J. H. Maiden, Director of the Sydney Botanic Gardens. The plant was collected at the beginning of 1920 by Mr. Allen, Curator of the Botanic Gardens of Darwin, in the Northern Territory. To both, the writer takes this opportunity of expressing his indebtedness and thanks.

The sample consisted of a small amount of air-dried leaves and a few beans.

Preliminary Tests.—An extract was prepared by macerating a small amount of the leaves, and also of the beans, in alcohol. When the solvent was distilled and the solid extract treated with slightly acidulated water, a solution was obtained which gave very strong indication of the presence of an alkaloid in considerable amount.

The alkaloid was contained in the leaves and, in relatively greater quantity, in the beans.

The characteristic tests for other active plant-principles gave negative results: cyanogenetic glucosides and saponins were proved to be absent.

The amounts of ash and water contained in the leaves and beans were estimated.

	Leaves.	Beans.
The air-dried powder contained water	8.73 %	10.1 %
„ „ „ ash	2.45	2.5

Extraction of the alkaloid.

The leaves and fruits were treated separately. The air-dried material was brought to a state of fine powder by passing through a grinding mill, and during this operation considerable discomfort was produced by the irritating effects of the dust on the mucous membranes.

The powdered plant material was transferred to large percolators and macerated with cold 70 % alcohol: this procedure was continued till the spirit drawn off ceased to contain alkaloid. In this way 24 litres of alcoholic extract were obtained, and this was distilled under reduced pressure at a temperature below 40°C.

The residue in the stills consisted of a thick black tarry mass, and this was poured into hot water. When the aqueous mass was left to settle a considerable quantity of resins was deposited, and this was washed repeatedly by decantation till the fluid no longer gave alkaloid reactions. The washings were concentrated and added to the main fluid.

This clear aqueous fluid was dark red in colour, slightly acid in reaction, and gave strong evidence of the presence of an alkaloid.

The faintly acid, aqueous fluid was then completely shaken out with ether in successive small volumes which removed colouring matter, chlorophyll, resins and fatty oils. This acid ethereal extract, measuring 10 litres, when distilled and dried, left a hard brown solid mass, weighing 20 gms.

The aqueous fluid was then made alkaline with sodium carbonate, and again extracted with ether, until the last ethereal solution contained no alkaloid. In this way the whole of the alkaloid was removed by the ether. The voluminous ethereal solution was distilled, the ether recovered, and the viscous syrup remaining in the still was transferred to a beaker. This residue which was dark brown in colour and resinous, was treated with faintly acidulated water, in which the alkaloid dissolved, leaving the resinous portion insoluble. From this acid solution the whole of the alkaloid was precipitated by sodium carbonate: it was then removed in solution by shaking up with ether, and the ethereal fluid distilled to dryness. This dry residue was pale amber-coloured and entirely amorphous, and when a second time it was extracted with acidulated water, sodium carbonate precipitated the alkaloid in white flocculent particles. These were removed, and dissolved in ether, from which the alkaloid was finally obtained as a white horny substance. It was then dried and weighed.

The yield of alkaloid.—From 2.8 kilograms of the leaves 56 milligrams of the amorphous alkaloid were obtained, or 0.002% of the air-dried leaf-powder.

The beans gave a much larger quantity—290 grams of this material yielded 87 milligrams of the alkaloid, or 0.03% of the air-dried beans.

Properties of the alkaloid.

Physical.—The substance obtained by evaporation of the ethereal solution from the leaves, and that from the beans, appeared to be identical, and consisted of a semi-transparent amorphous mass, almost white in colour.

It was soluble in alcohol, ether, ethyl acetate, chloroform, amyl alcohol and acidulated water, but quite insoluble in distilled water alone.

The solutions of the base exhibited a strong alkaline reaction and possessed an intensely bitter taste. The dilute acid solutions were readily and completely precipitated by sodium carbonate, or sodium hydroxide, while ammonium hydroxide

precipitated only concentrated solutions. These precipitates were white opaque flocculent masses, which on standing in the air for a short time became viscous.

Chemical.—Very dilute solutions of the salts of the alkaloid gave dense precipitates with the following characteristic reagents:—Wagner's solution, Mayer's solution, phosphotungstic acid, phosphomolybdic acid, picric acid, and tannic acid.

Sulphuric acid produced a bright yellow colour.

Potassium permanganate and concentrated sulphuric acid yielded a deep purple to red solution, somewhat similar to the strychnine reaction, with very slow reduction of the reagent.

Potassium bichromate and concentrated sulphuric acid rapidly produced a greenish blue colour which remained permanent.

The preparation of the hydrochloride of the alkaloid was next tried. About 40 milligrams of the pure dry amorphous alkaloid were dissolved in dry ether. Into this solution was passed a current of pure, dried, hydrochloric acid gas. A brown oily sediment gradually settled to the bottom of the vessel and on removal of the ether there was left a brown viscous residue which on examination proved to be the hydrochloride of the alkaloid. Many attempts were made to crystallise this substance, but it still retained its viscous nature. Nor were the efforts to transform it to sulphate and picrate salts more successful.

Determination of the chemical equivalent.—For this purpose a portion of the purified white amorphous alkaloid was dissolved in pure ethyl alcohol and water, treated with excess of centinormal hydrochloric acid, then carefully neutralised with centinormal soda and methyl orange indicator.

0.08 gm. alkaloid required for neutralisation	8cc. .01N	HCl
equivalent to	0.0029 gm.	HCl
100 gms. alkaloid would require	3.64	„
1000 gms. alkaloid would require	36.4	„
1 mol. wt. =	36.4	„

In formula [B].HCl equivalent weight of alkaloid = 1000 (approx.)
„ $[B]_2$.HCl „ „ = 500 „

Examination of the remaining solutions.

The ethereal extract, previously obtained by shaking out the acid solution with ether, was distilled, and from this 20 gms. of residue were obtained. This residue was redissolved in ether and treated with sodium carbonate solution. The alkaline liquid was agitated and run off a number of times, and these various solutions were notable for their brilliant colours, varying from *violet* to *crimson-red.* All these colours, however, soon became a uniform *reddish-brown,* and when the solutions were acidulated with hydrochloric acid, a dark brown oil formed on the surface, and a light *brown* curdy precipitate was deposited.

This precipitate was soluble in alkalies and alcohol, forming a deep *red* solution. Concentrated sulphuric acid also dissolved it as a bright *yellow* solution, which on dilution formed a *violet* precipitate; and ferric chloride produced an intense *green* colour.

The aqueous solution, after extracting the alkaloid with ether from the alkaline solution, possessed a deep *red* colour, was free from the bitter taste of the original solution, and gave no alkaloidal reactions. It was precipitated by lead acetate solution, and after the removal of the lead by hydrogen sulphide in the usual way, both precipitate and filtrate were examined.

The lead acetate precipitate.—The concentrated solution was shaken out with ether 10 times. This ethereal solution was a deep *yellow* colour and was next agitated with (*a*) ammonium carbonate, and (*b*) sodium carbonate.

(*a*). The ammonium carbonate extract was acidulated with sulphuric acid, shaken out with ether, and the solvent distilled. A buff-coloured crystalline residue was obtained, which was difficult to purify. This substance was soluble in water, alcohol, ether, chloroform, acetone and ethyl acetate, but these solvents dissolved both crystals and impurity together, and recrystallisation did not improve the appearance of the crystals. The aqueous solution was next digested with animal charcoal, and filtered. During the filtration the *colourless* solution became *purple*, the first washing with water yielded a *blue* solution, and the second washing became *green*.

The crystals which were obtained after this treatment were still impure. The aqueous solution was acid to litmus and possessed a hot peppery taste. It gave a negative reaction with Molisch's test. The crystals were lath-shaped with pointed ends and occurred in groups of rosettes. After drying at 100°C., the melting point was tested, when it was found that at 172°C. some change took place, resulting in the formation of a white sublimate in the tube, and a white film round each crystal. About 216°C. the substance melted and charred.

The neutralisation equivalent was obtained by titrating 14 milligrams of the crystals with 6.5 c.c. of centinormal soda, which gave 216 as the molecular weight of the acid. The amount of material was too small for further investigation.

(*b*). The sodium carbonate extract also exhibited the brilliant colours. For example, the first addition gave a bright *violet* (permanganate) colour to the alkaline solution, the second addition yielded a deep *green* colour which rapidly changed to *cherry-red*. Acidulated with sulphuric or hydrochloric acid the solution was *yellow*, and when reshaken with ether the ethereal solution also was a bright *yellow* colour. When filtered the latter solution became *red* and *violet* by oxidation. On evaporation the ether left a *red* solid. This substance was soluble in dilute ammonia forming a deep *reddish-violet* colour, gradually becoming *brown* on standing. This, on acidulating with sulphuric acid, gave an intense *yellow* colour.

The lead acetate filtrate.—This was shaken out with ether many times, and the ethereal solution after concentration was agitated successively with ammonium carbonate, sodium carbonate, and caustic soda. These solutions when acidified with sulphuric acid and agitated with ether, after distillation left a small oily residue of a dark brown colour.

Luteolin, and other colour substances.—These various alkaline liquids with the many different colours ranging from *yellow*, *green*, *blue*, to *violet* and *red* all gradually changed to a uniform *brownish-red* colour on standing for some time, and the acidulated solutions gave *brown* precipitates.

Certain of the above remarkable colour changes, and the reactions described, correspond to those obtained for the yellow dye *luteolin* by Power and Salway (19) in their investigation of *Erythrophloeum guineense*. These authors obtained a very small amount of luteolin from a large quantity of the plant, and they showed that it existed in the plant as a glucoside.

Conspicuous evidence of the presence of other powerful colouring matters was shown, but the amount obtained was not sufficient for further examination.

The aqueous solution remaining from the lead acetate filtrate after agitating with ether, contained much sugar.

PHYSIOLOGICAL ACTION OF *ERYTHROPHLOEUM LABOUCHERII.*

J. M. Petrie and H. Priestley, M.D., Ch.M., B.Sc., Associate Professor of Physiology.

(*a*) *External Action.*

The powdered leaves act as a violent irritant. During the grinding and drying of the material for analysis all those who came in contact with the fine dust, or inhaled the air of the room in which the powder was spread out to dry, suffered from violent fits of sneezing. In one case [J.M.P] the action of this irritating dust on the respiratory mucous membranes was so severe as to incite acute bronchial inflammation.

(*b*) *Action on cardiac muscle of the Frog.*

A solution of the purified hydrochloride salt of the alkaloid was prepared by dissolving it in 0.7% sodium chloride solution, that is, a physiological normal saline solution containing 0.5% of the alkaloid.

In a pithed frog the heart was exposed and the apex attached to a writing lever. After recording the normal beats for a short time the prepared alkaloid solution was dropped on the heart, from 2 to 4 drops being applied.

Exp. 1.—*Hyla aurea,* weight 12 gms.

Time	Heart-beats. per min.	Observations.
	64	Exposed heart beating regularly.
after		4 drops of alkaloid solution applied to heart.
5 secs.	70	
1 min.	58	Diastole incomplete.
6 mins.	24	Liver engorged, sinus venosus dilated.
		Convulsions
		Prolonged systole.
7 mins.		Heart stopped for 5 secs. in diastole.
10 mins.	0	Heart stopped in systole.
11 mins.		Fibrillar twitchings of the heart.

The diastolic standstill for 5 secs. was probably a result of stimulation of the vagus centre. Partial contractions of the auricles continued after the ventricle had stopped in complete systole.

Exp. 2.—*Hyla aurea,* weight 12 gms. (*See* diagram.)

Time	Heart beats	Observations
	48	Normal heart.
after		Applied 4 drops of alkaloid solution.
1 min.	48	
2 „	45	
3 „	38	Convulsive movements.
6 „	36	
7 „	0	Heart stopped, with ventricle slightly contracted.

In this instance when the heart stopped the ventricle was irregularly contracted, and it contained blood in small isolated patches, especially about the base. The diagram shows the record at the beginning, middle, and end of the experiment. The mark under the top line indicates where the alkaloid was applied.

Exp. 3.—*Hyla aurea*, weight 17 gms.

Time	Heart beats	Observations.
after	68	Normal heart. 4 drops alkaloid solution applied to heart.
1 min.	60	
2 „	54	Beats became weaker.
2½ „	30	Convulsive movements.
4 „	0	Heart stopped in systole.

The diastole became less perfect, and the systole stronger and more perfect.

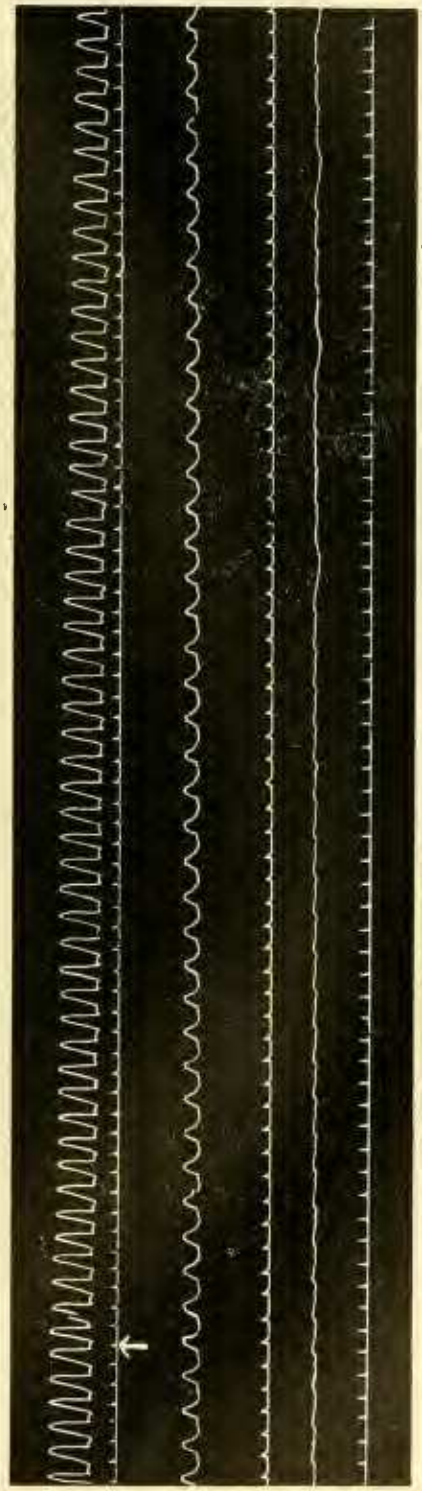
Exp. 4.—*Hyla caerulea*, weight 39 gms.

Effect of small dose on large frog.

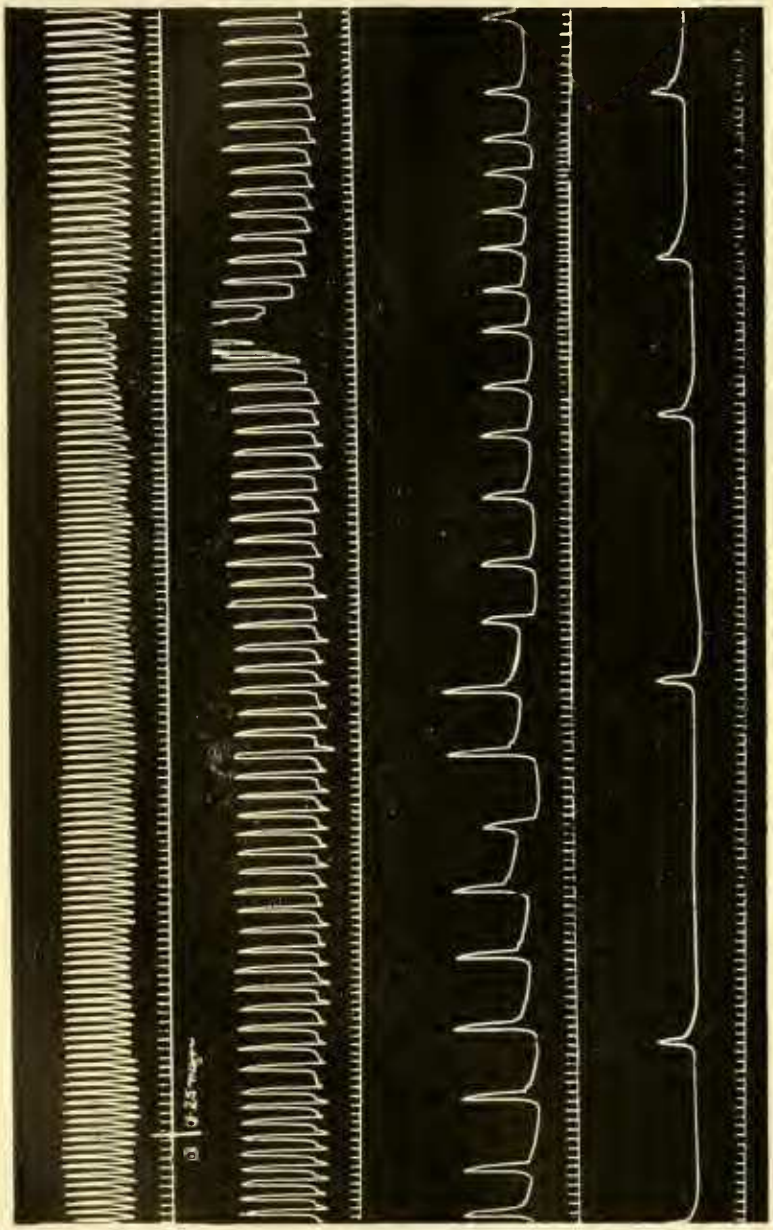
(*See diagram*).

Time	Heart beats	Observations.
	66	Normal heart.
after		1 drop alkaloid solution applied to heart.
1 min	63	
2 „	51	
4 „	63	
5 „	54	
6 „	57	1 drop alkaloid soln., 2nd. application.
7 „	51	
8 „	45	
10 „	30	
15 „	15	
20 „	9	Prolonged periods of stop in diastole.
29 „	1	Heart stopped in systole.
30 „	0	

Two drops of the 0.5% solution were applied in this case; the heart gradually beat slower and weaker, till it stopped with the ventricle in systole.



Exp. 2. The action of the Erythrophloeum alkaloid on frog's heart. (Top line, at start; second line, after 3 mins.; third line, after 7 mins.)



Exp. 4. Effect of 0·25 mgm. of alkaloid on frog's heart.

After the second application of alkaloid solution the rhythm of the heart changed. The systolic contractions were slightly increased. The beats were slower, and the continued widening of the curve, produced by the prolongation of the period of diastolic pause, was enormously drawn out near the end, till the final systolic contraction remained permanent.

In another experiment with the frog heart the number of beats decreased, and this was accompanied by an increase and lengthening of the diastolic phase. The ventricle remained full of blood when the heart had ceased to beat.

In the diagram, parts of this record are shown. The top line shows the commencement of the experiment, the second line after 10 minutes, the third line after 20 minutes, and the fourth line the end at 30 minutes.

(c) *Action on Dogs.*

The animals were anaesthetised by ether vapour. The blood pressure in the left carotid artery was recorded on the kymograph, and also the respirations by a stethograph drum fixed on the chest. The alkaloid, in the form of a saline solution of the hydrochloride, was injected from a burette into the right jugular vein. The concentration of this solution was equivalent to 1 milligram of alkaloid in each cubic centimetre of 0.9% physiological salt solution.

Exp. 5.—Dog, weight 3800 gms.

Time-interval	Blood-pressure	Respirations per min.	Heart-beats per min.	Observations.
	112 mms.	27	135	Normal records.
after				Ran in 2.5 mgs. of alkaloid during 16 secs.
20 secs.	184		150	Max. blood-pressure observed.
40 „	164			
60 „	176	26		
1 min. 40	132		81	Min. blood-pressure observed.
2 mins.	170	21		
3 „	176	22	140	
4 „	176	18		
5 „	176	14		
5½ „	176		140	
6 „	166	12	108	Blood-pressure began to fall.
7 „	156	14		
8 „	146	14	80	Heart-beats very irregular.
9 „		14		
10 „		11		
11 „		8		
12 „		6		
		0		

The time was reckoned from the completion of the injection.

The action of the alkaloid was almost instantaneous. 2.5 milligrams were injected, and before the last drop had entered the arterial pressure had risen considerably. The maximum pressure was reached 20 seconds after the end of the injection. The immediate result of the injection showed the blood-pressure

increased by 72 millimetres in 20 seconds and the rate of the heart beats increased by 15 per minute. The blood-pressure then fell and rose again, all within the first minute. After 1min. 40secs. the minimum point was reached where the blood-pressure dropped 52 millimetres below the maximum. It then rose, and remained high during 6 minutes, after which it began to fall, and continued till the end of the experiment.

The respirations decreased gradually from beginning to end. They had fallen to half the number in 6 minutes, and stopped at 12 minutes.

From the period of maximum blood-pressure, after 20 seconds the curve of respiration showed an ever increasing period of rest, or broadening out at the end of each inspiration.

In this experiment the intravenous injection of 0.0025 gram of alkaloid into a dog weighing 3800 grams proved fatal in 12 minutes. This amount is equivalent to 0.6 milligrams per kilogram of body-weight.

Exp. 6.—Dog, weight 2900 gms.

Time interval	Blood-press. in mms.	Respirations per min.	Observations.
	Mean 100	44	Normal records
after 1 min.	„ 135	47	Injected 1 mgm. alkaloid during 30 secs.
2 „	„ 115	50	
4 „	„ 135	50	Second injection, 1 mgm. alkaloid, 8 secs.
5 „		44	
6 „		40	
9 „	„ 135	44	
10 „	Max. 156		Third injection, 3 mgms. alkaloid, 18 secs., heart-beats 228 per min.
11 „	„ 180	40	
11½ „	„ 160	17	Blood-pressure began to drop.
12 „	„ 140	12	Heart-beats very irregular.
12½ „	„ 30	?	
13 „	zero	0	Fibrillar twitchings observed.

After the intravenous injection of 1 mgm. of alkaloid an instantaneous rise was observed in the arterial blood-pressure, which reached its maximum after 1 min. and then fell. After a second injection of the same amount the pressure rose again to the same level and this time remained up. After 10 minutes a third injection of 3 milligrams was made. This time, although the mean pressure was unchanged, the maximum height rose 24 millimetres. One minute after the last injection the pressure began to fall and 2 minutes later the animal was dead. The respirations after the first small dose were increased in number, but after each subsequent injection no increase was observed. The depth of respirations in this experiment did not alter to any extent.

A post mortem examination showed the heart in diastole, but congested with blood on the right side only. The right ventricle was extremely dilated while the left was empty.

The liver, spleen, and intestines were very pale in colour, and peristaltic movements were very conspicuous.

Exp. 7.—Dog, weight 9450 gms.

Action on Respiration.

Observations.	Time	Number of respirations per min.
Normal curve shows.		60
Injected 0.5 mgm. into artery.	after	
Blood-pressure rose.	1 min.	71
	2 „	71
	4 „	71
	5 „	63
Second injection 1 mgm. into artery.		
Blood-pressure rose.	6 „	60
	10 „	71
	15 „	85
Third injection 1 mgm. into artery.		
Blood-pressure dropped.	16 „	95
	17 „	79
Blood-pressure rose again.	18 „	71
	19 „	31
Heart-beats very irregular.	23 „	23
Dog dead.	25 „	0

The arterial pressure curve showed a rise of blood-pressure immediately following the first and second injections, but not the third. The last injection was followed by a sudden drop, and after 1.5 minutes by a rise to a still higher level.

Summary of Results.

When very small doses of the alkaloid were administered to animals, in these experiments, a complete change was observed in the heart-rhythm and respirations.

Blood-pressure.—During the few seconds required to run in the solution, the blood-pressure rose, and quickly reached a maximum. This was soon followed by a drop, which, however, never reached the previous normal level but rapidly rose again and remained high till near the end when it rapidly fell to zero.

Heart-beats.—In one frog alone the heart-beats were accelerated; in the other frogs the number was decreased. The dog in experiment 5 showed a large increase after the injection.

Respiration.—In experiment 5 after an injection of 2.5 milligrams the number of respirations gradually decreased during the 12 minutes, from 27 to 0 per minute. In No. 6 experiment 1 milligram injected produced an increase in number, but after a second and third injection the number gradually lessened, and ceased after 13 minutes. In No. 7 experiment 0.5 milligram accelerated the respirations, and after a second and third injection still further accelerations were observed. One minute after the last, the number decreased, and 7 minutes later the respiration ceased.

The chief characteristic of the drug is, therefore, its action on the heart-muscle. The tone is increased, heart relaxes less during diastole, and in the later stages the heart-beats become very irregular. The heart in most cases comes to a standstill in systole.

Convulsive movements were observed in the animals towards the end of the experiments.

The general result of these experiments would refer the alkaloid to the digitalis group in its pharmacological action.

Discussion of Results.

The alkaloids of E. Laboucherii *and* E. guineense *compared.*

(*a*) Chemical Properties.

The alkaloid of the Australian species closely resembles the description of Merck's pure erythrophleine. Both alkaloids and all their salts were unerystallisable syrups. The hydrochloride, prepared under the most careful conditions, was obtained as a viscous yellow oil, which dried into a brown solid glutinous mass: in this respect it resembled the product of Merck, and of Power and Salway.

Harnack's provisional formula for erythrophleine, $C_{28}H_{43}O_7N$, was obtained from the amorphous platinum salt, and represents a molecular weight of 505.

Power and Salway obtained an approximate agreement of the above formula from the analysis of the hydrochloride, which they prepared from a sample of Merck's erythrophleine. This hydrochloride salt yielded to the authors (6.9% Cl) 7.1% HCl and corresponds to the formula B.HCl (B=1 molecule of Base).

The alkaloid from the Australian species, on the other hand, when titrated with the greatest care gave 3.64% of hydrochloric acid, just half the amount obtained by Power and Salway. This amount, however, corresponds to the formula $[B]_2$ .HCl.

$[C_{28}\ H_{43}\ O_7\ N]$ .HCl requires 7.21% HCl.

$[C_{28}\ H_{43}\ O_7\ N]_2$ .HCl ., 3.61% HCl.

Accepting the latter as the formula of the hydrochloride obtained from the Australian *Erythrophloeum,* the agreement in the molecular weight thus found is so close as to justify the conclusion that the alkaloid is identical with the erythrophleine of the African species.

(*b*) Physiological Properties.

The violent effects produced on the respiratory organs by this plant, during the grinding and preparing of the sample for analysis, were also experienced and noted by all the investigators of *E. guineense.*

In the examination of a pharmacologically active plant, the collective effects produced in the animal body by the active principle are referred to certain types or groups of substances. In this way we observe the action of the atropine group, the curare, or the digitalis group. The action of the digitalis group is recognised chiefly by a special action on the cardiac muscle, with which there is a strengthening of the systolic phase of the heart and finally complete stopping of the ventricle in systole.

The substances which produce these effects are certain organic compounds, mostly glucosides, which have been obtained from plants, and include digitalis, apocynin, antiarin, convallamarin, helleborein, oleandrin, scillain and some of the African arrow-poisons.

The general action of the digitalis group as indicated above, was observed in all the experiments on frogs and dogs carried out with the alkaloid of *Erythrophloeum Laboucherii.*

The pharmacological properties were compared with those of *E. guineense* in the literature quoted, being the investigations of Lauder Brunton, Harnack, Merck, and Dr. Dale of the Wellcome Research Laboratories. The alkaloids of the two species of *Erythrophloeum* have the same action: that is, the active principle of the Australian *E. Laboucherii* is the alkaloid erythrophleine.

It is remarkable that a group of properties hitherto known to belong only to certain glucosides should also be exhibited by an alkaloid.

The red colouring substances.

Luteolin is a flavone derivative, one of a group of yellow dyes produced in the metabolism of plants. It has been identified in Dyer's weed (*Reseda luteola*), *Digitalis purpurea, Genista tinctoria* and *Erythrophloeum guineense.* It exists in three states:—as free luteolin, as methyl esters, and as glucosides of both of these. It is closely related to the quercetin dyes, including Mr. H. G. Smith's myrticolorin, obtained from the leaves of the Eucalyptus. Luteolin is isomeric with fisetin and lotoflavin, also yellow dyes. The latter constitutes one of the groups, with sugar and hydrocyanic acid, forming lotusin, the cyanogenetic glucoside of the lotus plants (*Lotus arabicus, L. australis, L. corniculatus,* etc.). All these flavone derivatives are built round the important pyrone ring, and by the number and position of their hydroxy groups their tinctorial properties are determined, and the various members are identified. Luteolin is a tetrahydroxy flavone.

A most prominent feature throughout this investigation was the deep red colour of the solutions. There is no doubt that this red colouring matter is closely connected with the characteristic colour of the wood, as myrticolorin is with the red stringybark (*Eucalyptus macrorhyncha*).

These flavone glucosides are important metabolic products of the plant tissues, and possess an astringent and very bitter taste. The intensely bitter taste of the original extract of the leaves of *E. Laboucherii* was doubtless in part due to this cause.

References to Erythrophloeum Laboucherii.

1. Bentham—*Flora Australiensis,* ii., 297; Engler, iii., 3, 127.
2. Mueller, 1859—*Journ. Linn. Soc. Lond.,* vol. iii., p. 159.
3. ————, Australian Acacias and allied genera (with fig.).
4. ————, Wittstein's organic constituents of plants (Eng. trans.), p. 331.
5. Bailey—Flora of Queensland, Part ii.
6. ———, Weeds and Poisonous Plants of Q., p. 47 (with fig).
7. ———, 1900—The Ironwood Tree. *Qland. Agric. Journ.,* vii., 1900, 153 (with fig.).
8. Bennett, F., 1904—Botany of Irvinebank, *Proc. Roy. Soc. Qland.,* xix., 1904, 65.
9. Planchon, L., 1907—Recherches sur les E. *Ann. Inst. Col. Marseille,* ser. 2, v., 1907, p. 161-304. through *Just's Botanischer Jahresbericht,* 1908, 36, abth. 1, s. 450.
10. Meston, A., 1909—A Remarkable Tree, *E. Laboucherii. Sydney Daily Telegraph,* Sept. 20th., 1909.

References to Erythrophloeum guineense.

11. Procter, 1856—*Erythrophloeum judiciale,* the sassy bark tree of Cape Palmas. *Pharm. Journ.,* 16, 1856, 233 (syn. *E. guineense*).

12. Gallois et Hardy, 1876—Recherches chimiques et physiologiques sur l'ecorce de Mancoue. *Bull. Soc. Chim. de Paris,* T. 26, 1876, 39; *Journ. de pharm. et de chemie,* 24, 1876, 25.
13. Brunton and Pye, 1876—On the physiological action of the bark of *E. guineense* or sassybark. *Phil. Trans. Roy. Soc.,* 167, 1877, p. 627.
14. See et Bochefontaine, 1880—Sur les effets physiologiques de l'erythrophloeine. *Comptes rendus de l'acad. Paris,* 90, 1880, 1366.
15. Harnack u Zabrocki, 1882—Untersuchungen uber das Erythrophloeine den wirksamen Bestandtheil der sassy-rinde. *Archiv. fur exp. Path. u Pharm.,* 15, 1882, 403.
16. Harnack, 1895—Berliner Klinische Wochenschrift, 1895, 759 (not seen).
17. Laborde, 1907—Etude chimique de l'ecorce d'*E. couminga. Ann. Inst. Col. de Marseille,* 2nd. ser. v., 1907, 305.
18. Merck, E., 1911—Digitalis glucosides and Allied Drugs. *Merck's Ann. Rept.,* 25, 1911, 93.
19. Power and Salway, 1912—Chemical Examination of the Bark of *E. guineense. Amer. Journ. of Pharm.,* 84, 1912, 337. *Well. Chem. Res. Labs.,* reprint No. 144.
20. Brill and Wells, 1917—Philippine Medicinal Plants. *Philipp. Journ. of Sci.,* 12, 1917, 167.

EXPLANATION OF PLATES XXIII.–XXIV.

Plate xxiii.

Erythrophloeum Laboucherii, leaves and fruit.

Plate xxiv.

Erythrophloeum Laboucherii.

Fig. 1. Close view showing characteristic bark.
Fig. 2. Tree growing in Northern Territory.